

***ADCK4* “reenergizes” nephrotic syndrome**

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J Clin Invest. 2013;123(12):4996-4999. <https://doi.org/10.1172/JCI73168>.

Commentary

Steroid-resistant nephrotic syndrome has a poor prognosis and often leads to end-stage renal disease development. In this issue of the *JCI*, Ashraf and colleagues used exome sequencing to identify mutations in the aarF domain containing kinase 4 (*ADCK4*) gene that cause steroid-resistant nephrotic syndrome. Patients with *ADCK4* mutations had lower coenzyme Q₁₀ levels, and coenzyme Q₁₀ supplementation ameliorated renal disease in a patient with this particular mutation, suggesting a potential therapy for patients with steroid-resistant nephrotic syndrome with *ADCK4* mutations.

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whereby specific circadian genes impact neuronal survival. Since we know that levels of BMAL1, CLOCK, and NPAS2 are tightly linked to light exposure and sleep deprivation, chronic circadian disruptions through activities such as shift work, travel, nighttime light exposure, or other environmental factors could have serious consequences for individuals predisposed to neurological disorders. Furthermore, these data indicate that those currently suffering from neurological disorders would certainly benefit from a stable sleep-wake and light-dark schedule. It will be interesting for future studies to determine whether any of these neuropathologies can be prevented through direct, local rescue of BMAL1 function or through manipulation of important clock target genes. Such studies might provide an avenue toward future therapeutic developments.

Acknowledgments

I would like to thank Ryan Logan and Trey Williams for helpful comments on this article.

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ADCK4 “reenergizes” nephrotic syndrome

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Steroid-resistant nephrotic syndrome has a poor prognosis and often leads to end-stage renal disease development. In this issue of the *JCI*, Ashraf and colleagues used exome sequencing to identify mutations in the *aarF* domain containing kinase 4 (*ADCK4*) gene that cause steroid-resistant nephrotic syndrome. Patients with *ADCK4* mutations had lower coenzyme Q₁₀ levels, and coenzyme Q₁₀ supplementation ameliorated renal disease in a patient with this particular mutation, suggesting a potential therapy for patients with steroid-resistant nephrotic syndrome with *ADCK4* mutations.

Steroid-resistant nephrotic syndrome

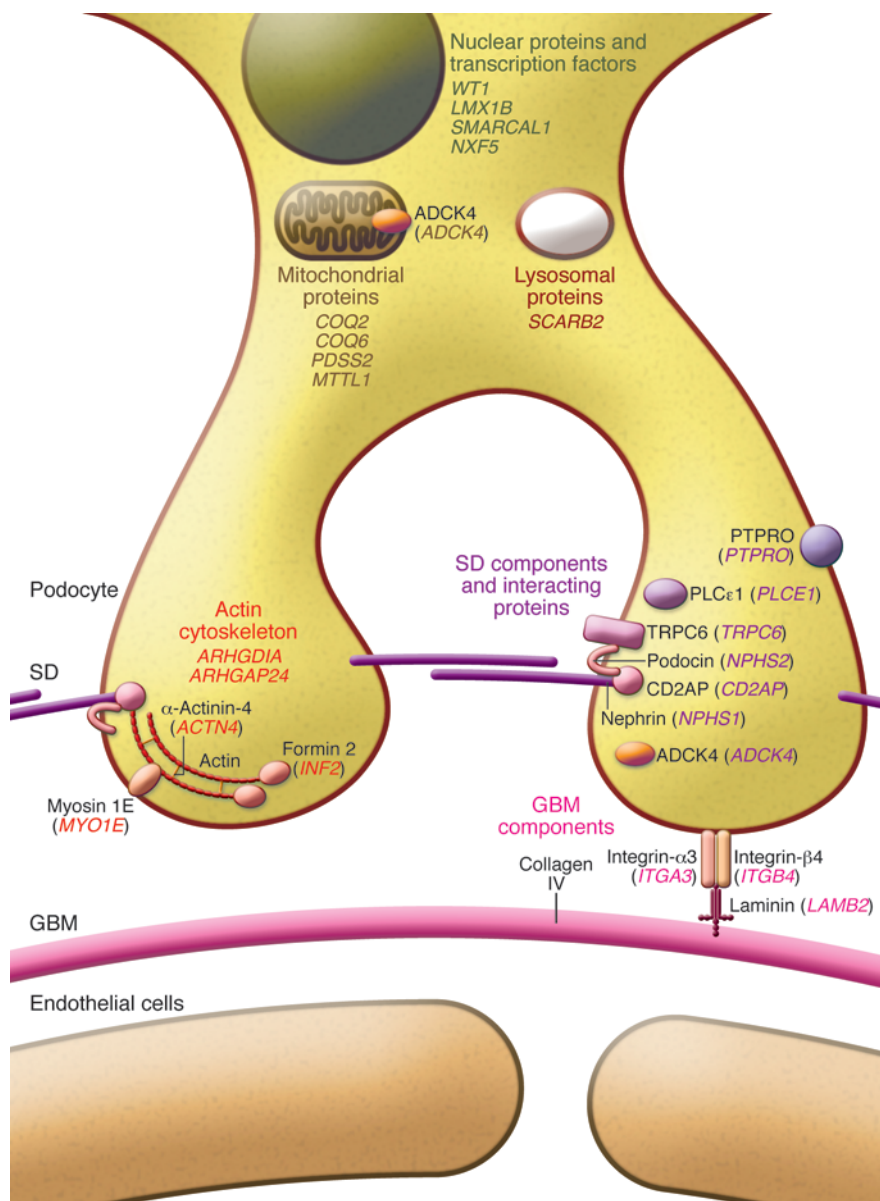
Nephrotic syndrome (NS) is the most common primary glomerular disease in children. All children with NS share similar clinical manifestations, such as edema, and biochemical abnormalities, including proteinuria, hypoalbuminemia, and hyperlipidemia; however, their clinical courses vary greatly (1). The majority of children presenting with idiopathic NS respond to steroid therapy.

Unfortunately, more than 20 percent of patients will fail to respond to steroid treatment. These patients with steroid-resistant NS (SRNS) usually progress to end-stage renal disease, necessitating dialysis or renal transplantation. Identifying patients who will respond to steroids and understanding the underlying disease pathogenesis is a critical challenge for disease management. Light microscopic lesions in children with SRNS overlap with those in children with steroid-sensitive NS. These changes range from focal segmental glomerulosclerosis and diffuse mesangial sclerosis to minimal change disease.

Podocyte foot process effacement observed by electron microscopy is the sine qua non of NS, indicating the role of these cells in NS development. Podocytes or glomerular epithelial cells cover the outer surface of the glomerular basement membrane and, along with the underlying endothelial cells, represent the glomerular filtration barrier. Mutations of more than 20 genes have been found to be associated with NS development in humans (Figure 1). Interestingly, all 24 genes associated with NS development are localized to the podocyte, further confirming that podocytes are essential in maintaining the glomerular filtration barrier. Mutations associated with NS can be divided into a few main groups. For example, mutations of podocyte actin cytoskeleton proteins are frequently found in adult SRNS or focal segmental glomerulosclerosis (2, 3). Podocytes appear to be dynamic cell types, and foot process remodeling is critical to maintaining the filtration barrier. Foot processes are connected by

Conflict of interest: Work in the laboratory of Katalin Susztak is funded by Boehringer Ingelheim.

Citation for this article: *J Clin Invest.* 2013; 123(12):4996–4999. doi:10.1172/JCI73168.

**Figure 1**

The products of the genes whose mutations have been found to cause SRNS reside in the podocyte. Mutations of these genes lead to abnormal or absent protein products. The complex ultrastructural nature of the podocyte makes it difficult to judge the exact function of each of these proteins on a molecular level and thus classify the different mutations. Slit diaphragm-associated (SD-associated) mutations (purple) include nephrin (*NPHS1*), podocin (*NPHS2*), PLC ϵ 1 (*PLCE1*), CD2-associated protein (*CD2AP*), transient receptor potential channel 6 (*TRPC6*), and protein tyrosine phosphatase receptor type O (*PTPRO*), and ADCK4 (orange). Cytoskeleton and foot process actin network-associated mutations (red) include α -actinin-4 (*ACTN4*), inverted formin 2 (*INF2*), myosin 1E (*MYO1E*), Rho-GDP-dissociation inhibitor 1 (*ARHGDI1A*), and Rho-GTPase-activating protein 24 (*ARHGAP24*). Nuclear- or transcription-associated mutations (gray) include Wilm's tumor protein (*WT1*), LIM/homeobox transcription factor 1 β (*LMX1B*), ATP-driven annealing helicase (*SMARCAL1*), and nuclear RNA export factor 5 (*NXF5*). Glomerular basement membrane-associated (GMB-associated) mutations (pink) include laminin- β 2 (*LAMB2*), integrin- β 4 (*ITGB4*), and integrin- α 3 (*ITGA3*). Mitochondria-associated mutations (brown) include parahydroxybenzoate-polyphenyl transferase (*COQ2*), ubiquinone biosynthesis monooxygenase (*COQ6*), decaprenyl-diphosphate synthase subunit 2 (*PDSS2*), mitochondrially encoded tRNA leucine 1 (*MTTL1*), and ADCK4. Lysosome-associated mutations (maroon) include scavenger receptor class B, member 2 (*SCARB2*).

a specialized adherens junction, the slit diaphragm. Over the last decade the slit diaphragm emerged as an important signaling platform (4). Mutations of slit diaphragm- and slit diaphragm-associated proteins represent another major group causing SRNS, especially in children (5). Molecular studies indicate that actin and slit mutations usually alter motility and morphology in vitro (6), causing foot process effacement in vivo. Morphological changes, including the foot process effacement and the associated NS, can be reversible in humans. Progressive glomerulosclerosis and end-stage renal disease develops when podocytes are lost due to apoptosis or detachment (7, 8). Podocytes

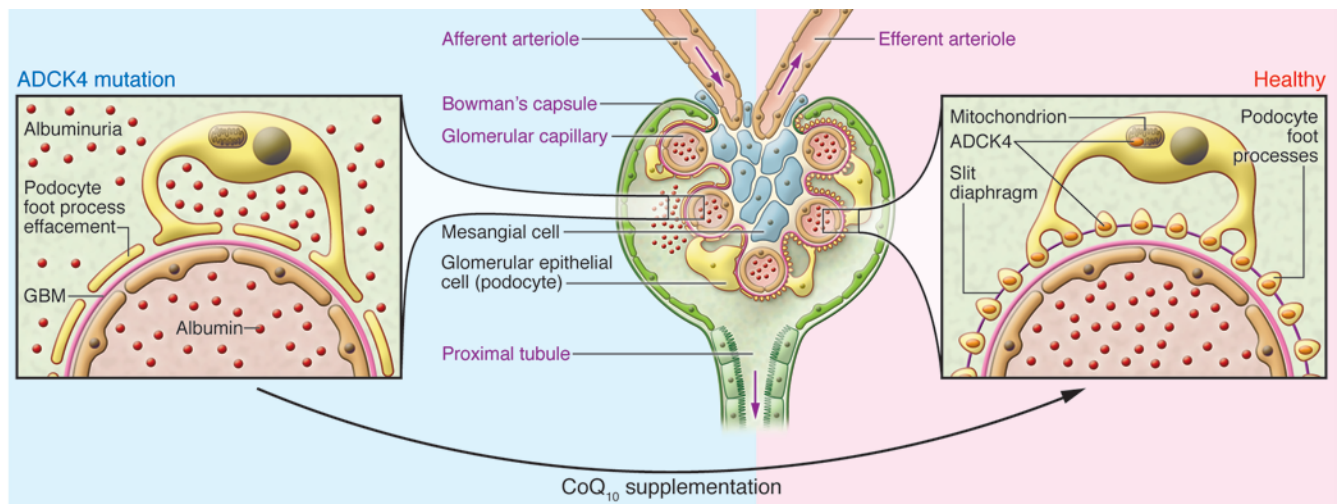
are unable to divide, so podocyte loss is associated with maladaptive hypertrophy and activation of repair pathways, further propagating podocyte damage and causing glomerulosclerosis (9, 10).

ADCK4 is implicated in NS

Whole-exome sequencing approaches revolutionized the identification of rare monogenic coding mutations associated with a strong penetrance (11). Exomes represent only 1.5% of the genome. Using next-generation sequencing, we can achieve sufficient coverage to identify specific mutations in coding regions.

In this issue of the *JCI*, Ashraf and colleagues coupled homozygosity mapping

with whole-exome sequencing in patients with SRNS to identify a unique disease-causing gene candidate: *ADCK4* (12). This gene is located on chromosome 19 and encodes the aarF domain containing kinase 4. The authors identified 11 different mutations in *ADCK4* in 15 individuals from 8 unrelated SRNS families. These mutations affected conserved amino acids. To determine whether *ADCK4* mutations truly cause NS, the authors reproduced the nephrosis phenotype in animal models. Knockdown of the *ADCK4* ortholog in zebrafish resulted in the characteristic edema, proteinuria, and microscopic changes of NS. In addition to the clinical phenotypes, the

**Figure 2**

Role of *ADCK4* in podocyte-dependent maintenance of the glomerular filter. The foot processes of WT podocytes are connected by a specialized adherens junction, the slit diaphragm. The complex interaction between podocytes and the glomerular capillaries prevents large molecules like albumin from escaping into Bowman's capsule. In WT podocytes, *ADCK4* (orange ovals) resides in mitochondria and in podocyte foot processes. *ADCK4* mutations are associated with foot process effacement and albuminuria (NS). CoQ_{10} supplementation reversed the phenotypic changes.

authors also noted podocyte foot process effacement and disorganization of the filtration slit, similar to that observed in patients with NS, indicating that *ADCK4* mutations are causally linked to NS.

A role for mitochondria in the pathogenesis of SRNS

The most frequently mutated SRNS-associated proteins are localized to the podocyte slit-associated signaling platform or the slit-associated actin cytoskeleton (Figure 1); however, *ADCK4* is slightly unusual. Ashraf and colleagues found that it localizes not only to the foot processes, but also to the mitochondria in rat podocytes (Figure 2). Additionally, they determined that *ADCK4* interacts with *COQ6* and *COQ7* proteins, which are involved in the coenzyme Q_{10} (CoQ_{10}) biosynthesis pathway. Levels of CoQ_{10} were decreased in individuals with *ADCK4* mutations, further suggesting that *ADCK4* is involved in CoQ_{10} biosynthesis. CoQ_{10} is an oil-soluble, vitamin-like substance present in most eukaryotic cells, primarily in the mitochondria. It is a component of the electron transport chain and participates in aerobic cellular respiration, generating energy in the form of ATP. Ninety-five percent of the human body's energy is generated this way. Therefore, those organs with the highest energy requirements, such as the heart, liver, and kidney, have the highest

CoQ_{10} concentrations. CoQ_{10} also acts as an antioxidant.

The mitochondria and CoQ_{10} are gaining momentum as key players in the development of renal disease. Mutations in three CoQ_{10} biosynthetic genes, *COQ6*, *COQ2*, and prenyl diphosphate synthase, subunit 2 (*PDSS2*), have been associated with SRNS due to dysfunctional mitochondria in podocytes (13–15). In mice with *PDSS2* mutations, administration of CoQ_{10} decreased albuminuria and interstitial nephritis (16). CoQ_{10} also ameliorated albuminuria and mitochondrial changes in an experimental model of type 2 diabetes (*db/db* mice) (17, 18). Importantly, Ashraf et al. also showed that CoQ_{10} was able to reverse the change in the podocyte migratory phenotype induced by *ADCK4*, indicating that mitochondrial dysfunction or reactive oxygen species generation is upstream of actin cytoskeleton changes. These changes appear to be clinically relevant, as the authors describe an isolated case of a patient with an *ADCK4* mutation who greatly benefited from CoQ_{10} supplementation. Low CoQ_{10} levels have been described in patients with different disease conditions, including hypertension, cancer, migraine, congestive heart disease (CHF), and Parkinson's disease. A clinical trial to test the effectiveness of CoQ_{10} in CHF is still ongoing. Unfortunately, the trial testing the role of CoQ_{10} in Parkin-

son's disease was stopped prematurely because of ineffectiveness. The study by Ashraf et al. indicates that CoQ_{10} supplementation may prove a successful therapy for focal segmental glomerulosclerosis in patients carrying *ADCK4* mutations.

Conclusion and future directions

Although polygenic diseases are more common than single-gene disorders, studies of monogenic diseases provide an invaluable opportunity to learn about the underlying molecular mechanisms of specific disease conditions. Mechanistic insight obtained from patients with rare forms of monogenic disease often can be applied for common disease conditions. Monogenic studies identified the podocyte as the key cell type causally related to NS development. The study by Ashraf et al. is especially significant, showing the critical role of *ADCK4*, podocyte mitochondria, and CoQ_{10} , which are potentially upstream of slit signaling and actin dynamics in SRNS. This represents a fresh new direction in NS and podocyte research. Future studies shall examine the role of podocyte mitochondria and CoQ_{10} in polygenic and secondary NS cases as well.

Acknowledgments

This work was supported by NIH R01DK076077, the Juvenile Diabetes Research Foundation, and the American Diabetes Association.



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Heavy LIFting: tumor promotion and radioresistance in NPC

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The epithelial-derived nasopharyngeal carcinoma (NPC) is a rare tumor in most of the world; however, it is common in southern China, northern Africa, and Alaska. NPC is often left undiagnosed and untreated until a late stage of disease. Furthermore, while radiation therapy is effective against this tumor, local recurrence due to radioresistance is an important clinical problem. In this issue, Liu et al. report on their identification of the IL-6 family cytokine leukemia inhibitory factor (LIF) as a serum predictor of local NPC recurrence following radiation therapy. The authors developed this initial finding to discover a role for the LIF/LIFR/mTORC1 signaling axis in NPC tumor cell growth as well as radioresistance.

NPC: a rare and difficult to diagnose epithelial cell cancer

Nasopharyngeal carcinoma (NPC) is a squamous-cell tumor that affects the epithelial cell lining of the nasopharynx. NPC is a rare tumor throughout the world, but it occurs with increased frequency in Southeast Asia and is tightly linked to EBV infection. While NPC can be cured by radiation therapy if diagnosed and treated early, often this cancer is not recognized until it has progressed to an advanced stage. Furthermore, approximately 20% of NPC patients have local recurrence follow-

ing radiation (1). Indeed, a common cause of local recurrence and poor survival in NPC is radioresistance. While new imaging approaches have improved diagnosis and survival rates, new approaches to identifying biomarkers that predict local recurrence will be important in mitigating NPC disease burden and mortality.

Identification of serum biomarkers for NPC local recurrence

A minimally invasive approach to screening NPC patients would be to identify molecules secreted from the tumor environment into the blood that could be used as clinically predictive biomarkers. Therefore, Liu et al. screened the serum of NPC patients with local recurrence and compared it with serum from those who had

gone into remission after radiation therapy (2). A panel of 20 cytokines was assayed, and a small group that included leukemia inhibitory factor (LIF), CXCL9, IL-10, IL-6, and SCF was among those differentially elevated in patients with local recurrence. Of the cytokines assayed, LIF was the most markedly different between NPC patients that responded to radiation therapy and those with local recurrence; therefore, LIF was further studied for its role in NPC pathogenesis and radioresistance.

Impressively, Liu and colleagues determined that LIF serum levels alone were predictive of NPC compared with healthy individuals (2). Furthermore, NPC patients with the highest levels of LIF were more likely to have local recurrence following radiation therapy; however, LIF levels were not predictive of either metastasis-free or overall survival. The authors also presented compelling immunohistochemical evidence that LIF levels in the tumor environment were higher than in normal tissues. Additionally, both LIF and the LIF receptor (LIFR) were overexpressed in NPC tumors as compared with adjacent tissue. These clinical observations suggested that LIF actually plays a role in NPC tumorigenesis rather than simply serving as a biomarker of local recurrence.

Conflict of interest: The author has declared that no conflict of interest exists.

Citation for this article: *J Clin Invest*. 2013; 123(12):4999–5001. doi:10.1172/JCI73416.